Chilling-injury of harvested tomato (Solanum lycopersicum L.) cv. Micro-Tom fruit is reduced by temperature pre-treatments

Kietsuda Luengwilai¹, Diane M. Beckles, Mikal E. Saltveit*

Department of Plant Sciences, University of California-Davis, One Peter Shields Avenue, Davis, CA 95616, USA

A R T I C L E   I N F O

Article history:
Received 31 August 2010
Accepted 26 June 2011

Keywords:
Tomato fruit
Chilling injury
Electrolyte leakage

A B S T R A C T

Heat-shocks were used to reduce the development of chilling injury symptoms during ripening of tomato fruit (Solanum lycopersicum L. cv. Micro-Tom). Mature green tomatoes were immersed in 30–50°C water for 3–9 min before being chilled at 2.5°C for 0.5, 1, 2, 3, or 14 days, and then held at 20°C for an additional 7–14 days. The affect of both heat-shock and chilling treatments were independent of fruit weight. Measured at 20°C after 14 days of chilling, fruit exposed to 40°C for 7 min exhibited reduced chilling injury symptoms, as measured by their advanced ripening score and decreased rate of ion leakage into an isotonic 0.2 M mannitol solution. Reduced rates of leakage from the symplastic compartment probably contributed to the 2-fold decrease in the amount of ions in the apoplastic space, when compared to the control. A subsequent paper will report the results of metabolic profiling of Micro-Tom tomato fruit subjected to treatments that significantly decreased their development of chilling injury symptoms.

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1. Introduction

Chilling injury (CI) is a physiological disorder of plants and plant organs caused by exposure to low, but non-freezing temperatures (ca. >10°C) (Raison and Lyons, 1986). Chilling-sensitive agriculturally important crops are often of tropical and subtropical origin and include avocados, bananas, soybeans, and tomatoes. The severity of injury depends on numerous intrinsic (e.g., cultivar, prior growing conditions, and prior exposure to stress) and extrinsic (e.g., temperature, duration of exposure, surrounding relative humidity, sanitation, and level of mechanical injury) factors. CI can occur before or after harvest, i.e., in the field, and during transport, storage and marketing (Morris, 1982). Symptoms are manifold, and include tissue browning, pitting and discoloration of the skin, uneven ripening, and increased disease susceptibility. These detrimental changes reduce quality and consumer acceptability leading to substantial economic loss.

Chilling can adversely affect harvested crops in two ways. First, exposure to low temperatures for certain durations can result in the development of chilling injury symptoms as described above. Second, since sensitive crops must be stored at temperatures above their chilling threshold, they cannot take full advantage of low temperature storage to slow metabolic activity and preserve quality.

Storage at these elevated temperatures shortens their market-life compared to crops that can be stored just above their freezing point. CI has been recognized, described, and studied for over 100 years (Saltveit and Morris, 1990), yet the primary cause and subsequent development of chilling injury symptoms remains unresolved. There is therefore both a practical need and an intellectual curiosity to understand the basis of this physiological disorder.

Conceptually, CI can be subdivided into two events; a primary event that is temperature-dependent and is initiated when the temperature falls below a threshold temperature for a specified duration, and causes some metabolic dysfunction. The secondary event is time-dependent and includes a multitude of metabolic processes that can be adversely affected as a consequence of the primary event, and lead to the development of measurable symptoms characteristic of chilling injury (Orr and Raison, 1990). Separating CI into these two stages helps to delineate the fundamental molecular mechanisms underlying this phenomenon, which is enormously complex. Moreover, it becomes possible to differentiate the primary ‘cause’ (i.e., the initial event happening upon chilling) from the secondary ‘effect’ (i.e., the subsequent events that produce physiological and visual signs of chilling injury) (Orr and Raison, 1990).

Heat-shock treatments reduce the development of chilling injury symptoms in tomato fruit (Saltveit, 2001). Exposure to temperatures at about 10–20°C above the normal growing temperature induces the production of a unique set of heat shock proteins (HSPs) that may function as molecular chaperones. These HSPs bind to unfolded or denatured proteins and prevent cell damage at low, chilling temperatures (Saltveit, 2002). In the experiments reported,
a heat-shock protocol is identified that reduces the development of chilling injury symptoms (i.e., preserves normal ripening, and decreases chilling-induced increases in ion leakage) in harvested mature green Micro-Tom tomato fruit.

2. Materials and methods

2.1. Plant growth conditions

Tomato (*Solanum lycopersicum* L. cv. Micro-Tom) seeds were a gift from Dr. David Weiss (The Hebrew University of Jerusalem, Israel). Tomato plants were grown from May to October 2009 in the Environmental Horticultural greenhouse, UC Davis, CA. Plants were grown in 15-cm diameter pots in standard soil media. Liquid fertilization with 100 mg/L nitrogen, 26 mg/L phosphorous, and 124 mg/L potassium was applied following standard cultural practices. Average daily PAR light in the greenhouses was 25.25 ± 2.62 mol m−2 s−1. Temperatures averaged 20 °C at night and 25 °C during the day. Humidity was approx. 50% during the day and 90% at night.

2.2. Fruit sampling and postharvest treatments

Mature green fruit (i.e., liquefying locular tissue, seeds not cut with a knife) (*Saltveit, 1991*) were hand-harvested between 7 and 8 am. Uniform and non-damaged fruit were washed in commercial bleach (1:20 dilution of 5% (v/v) sodium hypochlorite), and air-dried under a transfer hood. Three replicates of 4 fruit each were used for each of the 10 time points studied. Control fruit were held at 20 °C and heat-shocked fruit were immersed in a water bath at 30–50 °C for 3–9 min. All fruit were air-dried before being chilled at 2.5 °C for 0.5, 1, 2, 3, or 14 days. Fruit were then ripened at 20 °C for an additional 0.5, 1, 2, 7, 14 or 21 days. These experiments were repeated four times with slight variations in the duration of chilling exposure.

2.3. Ripening score

The degree of ripening was expressed as an average ripening score for each replicate. Ripening was scored as a visual determination of the surface color, where 1 = the surface completely green, 2 = less than 25% of the surface pink or red, 3 = more than 25% but not more than 60% of the surface pink or red, and 4 = more than 60% of the surface pink or red.

2.4. Respiration rate

The chilled fruit were removed from 2.5 °C and held at 20 °C for 1, 4, 8 or 24 h before respiration rate was measured. Four fruit per replicate were placed into a sealed 500 ml glass container for 1 h. A 1 ml sample of the head space was withdrawn using a syringe and its CO2 concentration measured with an infrared gas analyzer as previously described (*Saltveit and Strike, 1989*). Fruit were then dissected for subsequent study.

2.5. Dissection of fruit

A 1 cm diameter stainless steel cork borer was used to remove the central core from each fruit. The bottom pericarp disk thus excised was rinsed in deionized (DI) water to remove adhering tissue and immediately frozen in liquid nitrogen and kept at −80 °C until used for metabolic profiling (to be reported in a subsequent paper). The remaining locular tissue was cut into four radial segments and washed of adhering tissue with DI water. One radial segment from each of four fruit (one replicate) was placed into each section of a 4-sector 25 × 100-mm diameter plastic Petri dish. Each of the four sections thereby contained one radial segment from each of the four fruit per replicate, and each dish contained all the radial segments from the four fruit in a replicate.

2.6. Ion leakage measurement

The dishes with the excised radial segments were placed into plastic tubs lined with wet paper towels and loosely covered with aluminum foil. The tubs were held at 12.5 °C for 18 h to produce ‘aged’ tissue, i.e., to overcome the wound-induced alterations in membrane permeability (*Saltveit, 2005*).

After transferring to room temperature (approx. 18 °C), the four aged segments from each sector of the Petri dish were put into a 50 ml plastic centrifuge tube containing 20 ml of an aqueous solution of 0.2 M mannitol. It had previously been determined that 0.2 M was isotonic for this tissue (*Saltveit, 2005*). The conductivity of the bathing solution was measured with an Extech Model 480 digital conductivity meter (Waltham, MA) every 5 min for 30 min and then less frequently for 180 min with gently shaken between readings. After 3 h the tubes were capped, frozen at −20 °C and warmed to room temperature and frozen and thawed twice before the total conductivity of the solution was measured at room temperature after 1 h of shaking.

3. Results and discussion

3.1. Response of harvested mature green fruit to chilling

Micro-Tom tomato fruit have been used as a model system to study chilling injury (*Neta-Sharir et al., 2005; Malacrida et al., 2006; Gomez et al., 2009; Weiss and Egea-Cortines, 2009*). The ripening score of mature green fruit held at 20 °C increased linearly (*R2 > 0.93*) from 7 to 21 days after harvest (Fig. 1). The fruit were harvested at the mature green stage and developed ripening scores of 2.3, 3.6 and 4.0 after 7, 14 and 21 days, respectively. In contrast, chilling the mature green fruit at 2.5 °C for 0 to 3 weeks produced a linear decrease (*R2 > 0.99*) in their ripening score measured after holding the chilled fruit at 20 °C for an additional 14 days (Fig. 2). The ripening score decreased 15% (from 3.3 to 2.8) after one week of chilling, but it was not significantly different from that of the non-chilled control (*P = 0.05*). Extending chilling for 2–3 weeks produced a significant 35% and 2-fold decrease in the ripening score, respectively. Two weeks was selected as the duration of chilling because fruit could recover from this modest level of injury and treatments that modulated the fruit’s chilling tolerance would produce a greater change in the response (i.e., changes in the ripening
score and ion leakage) than applying the same treatment to fruit that were exposed to an excessive level of chilling.

3.2. Response of harvested mature green fruit to heat-shock and chilling

Mature green fruit were heated to various temperatures for various durations before being chilled at 2.5 °C for two weeks and then ripened at 20 °C for 7 days (Fig. 3). The ripening scores of fruit heated to 40 or 45 °C for 7 min (2.7 and 2.4, respectively) were significantly higher (110% and 85%, respectively) than the control fruit (a score of 1.3) or all but one of the other treatments (Fig. 3). Since the 45 °C treatments produced visual damaged on some fruit, the 40 °C for 7 min treatment was selected for further study.

3.3. Effect of fruit size (fresh weight) on response to heat-shock and chilling

There was no effect of fruit size (i.e., fresh weight) on the ability of heat-shock treatments to reduce chilling induced retardation of ripening (Fig. 4). Fruit were divided into 3 groups based on fresh weight. Small fruit weighed 2.5–4 g, medium fruit weighed 4–6 g and large fruit weighed 6–7.5 g. Fruit held at 20 °C (i.e., no heat-shock) or heat-shocked (30–50 °C) showed similar rates of ripening regardless of fruit size (R² = 0.0002). However, in all subsequent experiments fruit were carefully selected for uniform age and size (i.e., 4–6 g) to minimize possible variation in responses among fruit.

3.4. Effect of heat-shock and chilling on water loss

Heat-shock treatments did not affect weight loss (Fig. 5). Pitting and shriveling are visible symptoms of CI that are caused by increased rates of weight loss. Fruit were weighed after heat-shock treatments, after chilling at 2.5 °C for 14 days, and after holding at 20 °C for an additional 7 days. There was no significant difference in the rate of water loss from fruit subjected to any of the treatments (data not shown). However, when the rate of water
loss was expressed as grams per surface area (g/cm²), there was still no significant differences among the heat-treated fruit immediately upon removal from chilling, but when held for an additional 7 days at 20 °C, there was a slight, but not significant, increase in the rate of water loss with increasing treatment temperature. The rate of water loss from chilled fruit previously shocked at 45 °C for 7 min was significantly higher than from non-heat-shocked and non-chilled fruit. The 45 °C heat-shock treatment may have altered the structure and permeability of cuticular waxes (Roy et al., 1994). Although the rate of water loss was higher from heat-shocked fruit, it was still below the threshold that would reduce market quality (Kays, 1991).

3.5. Response of heat-shock treatment and chilling on respiration

The selected heat-shock treatment (40 °C for 7 min) reduced the transitory burst in respiration from chilled tomato fruit transferred to 20 °C. Chilled fruit exhibit an increase in respiration upon warming (Lyons and Breidenbach, 1990). This increase may be a consequence of the need for more energy to repair cellular damage from chilling. Treatments that reduce the development of chilling injury would therefore reduce the need for this added energy, and thereby reduce the increase in respiration following chilling.

A typical, modest respiratory climacteric was observed in fruit that ripened at 20 °C. Respiratory rates increased from a pre-climacteric rate of 37 mg CO₂ kg⁻¹ h⁻¹ on days 2–4 after harvest to 92 mg CO₂ kg⁻¹ h⁻¹ on day 5, before decreasing to 46 mg CO₂ kg⁻¹ h⁻¹ as the fruit continued to ripen on days 6–7 (Fig. 6). Holding the fruit at 2.5 °C for 14 days prevented ripening and this modest climacteric rise in respiration.

Respiration of non-heat-shocked fruit was similar to that of heat-shocked fruit for the first 4 h at 20 °C following 14 days at 2.5 °C (Fig. 6, insert). Respiration from the non-heat-shocked fruit subsequently increased to twice that of heat-shocked fruit after 8 h at 20 °C, before returning to levels similar to those of non-heat-shocked fruit (and to the non-climacteric levels of the non-chilled fruit) for the remainder of the 6 days at 20 °C (Fig. 6). If the dramatic rise in respiration at 20 °C following chilling represents the increased requirement of metabolic energy to repair chilling injury, then the heat-shock treatment apparently reduced the extent of chilling injury and the level of metabolic activity necessary to produce the needed repairs.

3.6. Effect of heat-shock and chilling on the rate and kinetics of ion leakage

The rate of ion leakage did not change significantly for either temperature treatment when measured at 20 °C after up to 3 days of chilling (3.3 ± 1.1% total conductivity per hour) (Fig. 7A). However, after 14 days of chilling, the rate of ion leakage from control (non-heat-shock) fruit was significantly higher than from heat-shocked fruit. Total conductivity per hour increased 3.3-fold to 14 ± 0.1%, and 2.4-fold to 8.0 ± 1.2% from control and heat-shock fruit, respectively. The rate of ion leakage decreased from fruit tissue held at 20 °C after chilling, indicating the tissue was able to recover from chilling injury for both temperature pre-treatments. Leakage from heat-treated fruit returned to the pre-chilling level within 1 day at 20 °C, while leakage from control fruit remained significantly higher.

A kinetic analysis of ion leakage from fruit subjected to either temperature pre-treatment produced results that were similar to those previously report (Saltveit, 2005). In the analysis, the coefficient Kf describes the rate of ion leakage from the apoplastic compartment (i.e., outside the cellular membrane) while the coefficient Ks describes the rate of ion leakage from the symplastic compartment (i.e., across the cellular membrane).

The two coefficients remained relatively constant during the first 3 days of chilling (Fig. 7B and C). After 14 days of chilling, Kf from control fruit was similar to the 3-day value while it was 30% less from heat-shocked fruit tissue. It remained stable from the heat-shocked tissue, while it decreased from the control fruit tissue to levels similar to that from the heat-shocked fruit tissue within 1 day at 20 °C. In contrast, Ks increased 6-fold and 2-fold from non-heat-treated and heat-treated fruit, respectively, after 14 days of chilling. Again, both values decreased during the 1 day at 20 °C, but Ks from the non-heat-shocked fruit tissue remained significantly higher that from the heat-shocked fruit tissue at the end of a 1 day holding period at 20 °C.

A similar decline in Kf and a small increase in Ks were reported from excised tomato pericarp tissue (Saltveit, 2005). Changes in Ks were interpreted as reflecting membrane repair in the chilled fruit tissue. Remember that the excised pericarp tissue had been ‘aged’
is no large concentration gradient as there is with excised tissue immersed in an isotonic mannitol solution, the net flux should be close to zero. So the concentration of ions in the apoplastic compartment will be a direct result of leakage and uptake of ions through the semi-permeable cellular membrane. If chilling caused an immediate increase in membrane permeability, there should have been a predominante rise in Cf with time of chilling. However, Cf declined during the first day of chilling and remained constant for the next 2 days for both the non-heat-shocked and heat-shocked tissues. Only when warmed to 20 °C after 14 days of chilling was there a rise in Cf. As with the other kinetic descriptions of ion leakage, the value for Cf was higher for the non-heat-shocked tissue immediately after chilling, but recovered and was similar to the heat-shocked tissue for the remainder of the recovery phase at 20 °C. The declining value of Cf for chilled tissue from both treatments indicates that the rate of ions absorption by the cells was greater than the rate of leakage from the cells. The smaller values immediately after 14 days of chilling for Kf, Ks and Cf indicate that the heat-shock treatment had a significant effect on the development of one of the most pronounced symptoms of chilling injury; namely the chilling-induced increase in membrane permeability.

3.7. Effect of heat-shock treatment and chilling on change in metabolite levels

Heat shock treatments may protect tissues from CI by reducing the synthesis of metabolites that contribute to the development of CI symptoms (e.g., toxic compounds, reactive oxygen species, etc.) (Salvit, 2001). Recent advances in metabolic profiling have led to the identification of novel metabolites and pathway associated with adaptation to low temperature stress (Cook et al., 2004; Hannah et al., 2006).

It is important to note that changes in metabolite levels do not always indicate the induction or presence of an adaptive pathway. Changes in metabolite pools could be a stress response caused by an imbalance in metabolism (i.e., chilling injury). Therefore, it is critical to distinguish between changes that are adaptive and changes that are part of the dysfunction of chilling injury. To differentiate between adaptive and dysfunctional chilling responses, we will compare physiological and metabolic changes in heat treated-fruit (with increased tolerance to CI) and non-heat treated-fruit (that were fully susceptible to CI), both before and after chilling. The metabolites that change in the CI-tolerant fruit, but not in susceptible fruit may be associated with the tolerance mechanism. The metabolites that change in the susceptible fruit but not in the tolerant fruit may be part of secondary events leading to the visible CI symptoms (Beckles and Roessner, 2011). Results of this detailed metabolite profiling will be presented in a subsequent manuscript.

Acknowledgements

The Anandamahidol Foundation supported K.L. This GC–MS profiling was funded by a pilot grant from the Metabolomic Core Genome Center, UC Davis and Henry A. Jastro Research Scholarship Awards (K.L).

References


Fig. 7. Effect of heat-shock treatment and chilling on the ion leakage from the same fruit as in Fig. 6. The rate of ion leakage (A), coefficients of fast compartment, Kf indicating the ion leakage from apoplastic compartment (B), coefficients of slow compartment, Ks indicating the ion leakage from symplastic compartment (C) and the concentration of ions in the apoplastic compartment (D). All kinetic parameters were calculated as described in the text. Values are mean ± S.D. of 12 fruit.

for 18 h at 12.5 °C prior to measuring ion leakage, thereby possibly giving it time to recover from some of the detrimental effects of chilling. Measurements of ion leakage were not done immediately after excision as wounding has a significant effect on membrane permeability and could have significantly affected the rates of ion leakage measured (Salvit, 2002).

Since Kf measures the efflux of ions from a volume not bounded by a semi-permeable membrane (e.g., the cell wall), chilling-induced changes to membrane permeability should have the minimal effect observed. In contrast, Ks measures the rate of ion efflux from membrane bounded volumes (i.e., cells) and is 500-times smaller than Kf. At the beginning of the chilling exposure, the average value for Kf was 0.25, while the average value for Ks was around 0.0005; a 500-fold difference.

The total conductivity from the apoplastic compartment Cf increased 2.0-fold and 1.3-fold in control and heat-shock fruit, respectively, after 14 days of chilling (Fig. 7D). In the intact fruit, Kf reflects the ability of ions to move within the tissue, but since there